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Saskatoon and Wild Blueberries Have Higher Anthocyanin Contents than Other Manitoba Berries

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Studies have shown that anthocyanins present in berry fruits have some beneficial health effects such as reducing age-associated oxidative stress and possessing anti-inflammatory properties. Therefore, six Manitoba berries (wild blueberry, Saskatoon berry, raspberry, chokecherry, strawberry, and seabuckthorn) were studied for their anthocyanin compositions (mg/100 g) on dry weight basis. Saskatoon berry and wild blueberry showed a high content of total anthocyanins (562.4 and 558.3 mg/100 g, respectively) that were not significantly ($P > 0.05$) different from each other. The corresponding values for other berries: raspberry (365.2 mg/100 g), chokecherry (177.39 mg/100 g), and strawberry (97.5 mg/100 g) were significantly different from each other ($P < 0.05$), and the total anthocyanin content of seabuckthorn was negligible (0.84 mg/100 g). Fifteen major anthocyanins were isolated from Manitoba berries. Saskatoon berry and wild blueberry contained higher amounts of delphinidin 3-glucoside (Dp-3-glc), malvidin 3-glucoside (Mv-3-glc), and malvidin 3- galactoside (Mv-3-gal). Dp-3-glc was 263.8 (mg/100 g) in Saskatoon berry and 84.4 (mg/100 g) in wild blueberry, whereas the corresponding values for Mv-3-glc in these berries were 47.4 and 139.6 (mg/100 g), respectively. Raspberry, strawberry, and chokecherry contained higher amounts of cyanidin 3-glucoside (Cy-3-glc), cyanidin 3-rutinoside (Cy-3-rut), and pelargonidin 3-glucoside (Pg-3-glc). The total anthocyanin content of Manitoba fruits followed the order: Saskatoon berry and blueberry (high anthocyanin berries), raspberry and chokecherry (medium anthocyanin berries), strawberry (low anthocyanin berries), and seabuckthorn (negligible anthocyanin berries). This study demonstrated that Saskatoon berries and wild blueberries have high potential value for fruit growers as well as the food and nutraceutical manufacturers because of their high anthocyanin contents.

INTRODUCTION

Berries have been shown to have a potential in the nutraceutical and functional food market (*1–4*). Berries are rich sources of essential micronutrients, particularly vitamin C (ascorbic acid), and folic acid (*5, 6*). They also contain proanthocyanidins (condensed tannins) that have potential health benefits such as antioxidant, anticarcinogenic, and anti-inflammatory effects (*6–10*).

Anthocyanins are pigments that give red, violet, and blue colors in most fruits, vegetables, and cereals (*11*). **Figure 1** shows the basic structure of anthocyanins. In plants, anthocyanins occur in a 3- or 3,5-glycosylated form of anthocyanidins (aglycones), generally linked with glucose, galactose, arabinose, rhamnose, xylose, or fructose (*12, 13*). The main anthocyanins in fruits are glycosides of six anthocyanidins. Cyanidin is the predominent anthocyanidin, followed by delphinidin, peonidin, pelargonidin, petunidin, and malvidin (*14–16*). Delphinidin is known to be responsible for the bluish colors, whereas cyanidin and pelargonidin are responsible for red and purple colors, respectively. At present, the most satisfactory method for analysis of mixtures is the multistep method of separation, isolation, and quantification by LC with peak identification by MS (*17*). Although organic solvents such as acidified water, methanol, and acetone are generally used for extraction of anthocyanins (*12, 17–19*), acidified MeOH exhibited the highest extraction efficiency (70–100%) (*17, 18, 20*).

Anthocyanins have been associated with antioxidant and anti-inflammatory properties (*6, 21*). They are capable of free-radical scavenging and chelation of trace metals, and they can reduce lipid peroxidation and DNA oxidation. Anthocyanins (*21, 22*), resveratrol (3,4′,5-trihydroxy-*trans*-stilbene) (*22, 23*), and other phenolics have been reported to contribute to the phenomenon called the "French Paradox". The French paradox was reported for the first time in 1819 and refers to the fact that people in France have relatively low incidence of coronary heart disease (CHD), despite consumption of a diet rich in saturated fats (*21, 22*). The major objective of this study was isolation,

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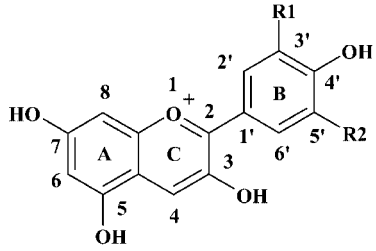

Aglycone	R1	R2
Cyanidin (Cy)	OH	H
Peonidin (Pn)	OCH3	H
Pelargonidin (Pg)	H	H
Malvidin (Mv)	OCH3	OCH3
Dephinidin (Dp)	OH	OH
Petunidin (Pt)	OCH3	OH

Figure 1. Basic structure of the anthocyanins.

identification, and quantification of major anthocyanins in six different Manitoba berry fruits.

MATERIALS AND METHODS

Six Manitoba fruits (strawberry, Saskatoon berry, raspberry, wild blueberry, chokecherry, and seabuckthorn) were collected at their peak of ripeness, transported to our laboratory within 12 h of picking, chilled on route and upon receipt, and freeze-dried in a Virtis Genesis freeze-dryer (SP Industries, Gardiner, NY). Berries were hand-picked during the period from June to August of 2006 from orchards surrounding Winnipeg (Manitoba, Canada). Strawberries, raspberries, and chokecherries were harvested when they were completely red and fully ripened. Wild blueberry and Saskatoon berries were harvested when completely dark-blue in color. Seabuckthorn berries were harvested via cuttings when they were fully ripe and bright-yellow in color. Each of the berries came from one variety. The fruits were randomly picked from several trees in the orchard and then combined for further analyses. The analyses were conducted on the dry powders obtained after grinding the freeze-dried samples. The data were expressed on a dry weight basis.

Chemicals. The solvents, methanol, acetonitrile, and acetic acid, were HPLC grade (Fisher Scientific Co., Ottawa, ON). The standards, delphinidin 3-glucoside (Dp-3-glc), malvidin 3-glucoside (Mv-3-glc), cyanidin 3-glucoside (Cy-3-glc), cyanidin 3-rutinoside (Cy-3-rut), and pelargonidin 3-glucoside (Pg-3-glc), cyanidin 3-galactoside (Cy-3-gal), delphinidin 3-rutinoside (Dp-3-rut), peonidin 3-glucoside (Pn-3-glc), and cyanidin chloride (Cy-Cl) were purchased from Polyphenols Laboratories AS (Sandnes, Norway). The standards were dissolved in acidified MeOH (1 N HCl, 85:15, v/v) to obtain concentrations of 1 mg/ml each.

Extraction and Purification. The extraction and purification of anthocyanins were accomplished according to a modification of the method of Naczk and Shahidi (*24*), as summarized in **Figure 2**. To determine the recovery of anthocyanins, fruit samples were also spiked with standards prior to extraction.

The extraction apparatus included a shaker (G-25, Eberbach, Ann Arbor Corp, Michigan, USA), centrifuge (SLA-3000) with Sorvall GS-3 rotor (Sorvall Instruments, Ontario, Canada), and rotary evaporator (Yamato RE-51, Cole-Parmer Instrument Company, Illinois, USA) with a water bath (Thermo-Lift, Fisher Scientific, New Jersey, USA).

HPLC Analysis. The HPLC method for anthocyanin measurements was based on modification of methods reported earlier (*17, 24*). Analyses were conducted on a HPLC (Waters 2695) system equipped with a photodiode array detector (Waters 996), Empower software, and autosampler (Waters 717 plus, Waters Corp., Milford, MA). The separation of anthocyanins was done at three different temperature (23, 35, and 40 °C) on a Luna 3u C18 column (150 × 3 mm i.d., 3 um) with a guard column (Phenomenex, Torrance, CA). An optimum temperature of 35 °C was selected. Mobile phase A was 4.5−6% formic acid or acetic acid in double deionized water, and mobil phase B was 100% methanol. Better resolution was obtained using formic acid instead of acetic acid. Furthermore, 6% formic acid performed better as compared to the 4.5% formic acid previously reported (*18, 25*). The gradient condition was as follows: solvent B: 0 min, 10%; 30 min, 25%; 40 min, 45%; 42 min, 90%; 45−50 min, 10%. Spectral data (254–600 nm) was collected for all samples. Elution of compounds of interest was monitored at a wavelength of 520 nm for anthocyanins and at 280 and 320 nm for other phenolics. Other chromatographic conditions were as follows: flow rate, 0.4 mL/min; injection volume, 10 μL; and a run time of 50 min. Peak identification of each anthocyanin was based on comparison of relative retention time (RT), percentage peak area, and spectral data with those of anthocyanin standards. The content of each anthocyanin was measured based on the area of the sample to the area of the corresponding standard. Cy-3-glc was used as an external standard to calculate the content of those anthocyanins that lacked standards. Under the current chromatographic conditions, the limit of detection (LOD) and limit of quantification (LOQ) were determined to be 100 ng/mg (S/N > 5) and 200 ng/mg (S/N > 10), respectively.

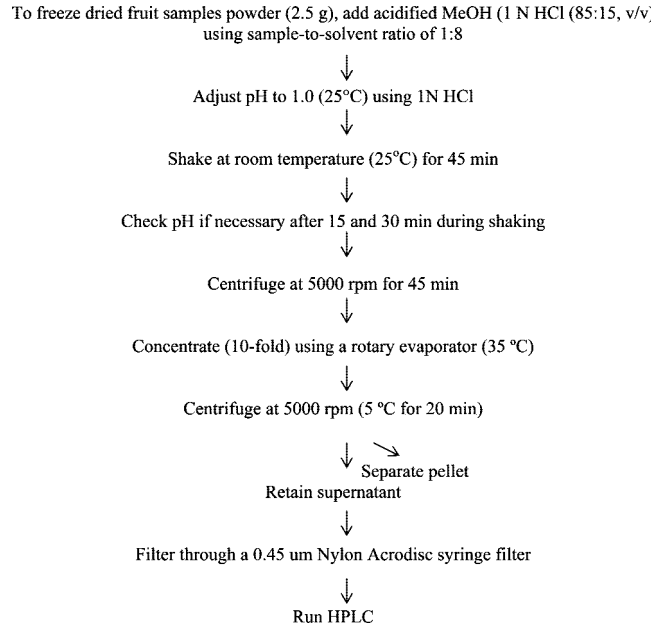

Figure 2. Extraction method for anthocyanins in berries.

Ultra Performance Liquid Chromatography (UPLC) Coupled with ESI-MS/MS. LC separation was performed on an ACQUITY UPLC system consisting of a binary pump, a sample manager, and a PDA detector set at 280 nm (Waters Corp, Milford, Massachusetts, USA). The ACQUITY UPLC BEH C18 column, 1.0 × 100 mm, 1.7 μm, was used for separation of anthocyanins (0.2 mL/min). The solvents were 0.1% formic acid (solvent A) and 100% methanol (solvent B). Prior to mass spectrometric (MS) analysis, a binary mobile phase consisting of 0.1% formic acid (A) and 100% methanol (B) was used under the following gradient conditions: 0–8 min, 13–24%B; 8–12 min, 24%B; 12–22 min, 24–100%B, followed by a 4 min re-equilibration of the column before the next injection. The eluting stream from the UPLC was introduced into a Waters Quatro Micro API mass spectrometer (Waters Corp, Milford, Massachusetts, USA) equipped with an ESI Multi-Mode Ionization probe (ESI APCI). We optimized the MS parameters using the anthocyanin standards. All spectra were obtained in both positive- and negative-mode ESI, and the scan was set at *m/z* 100–1900. MS parameters were as follows: capillary voltage, 3 kV; cone voltage, 30 V; extractor voltage, 3.3 V; source temperature, 100 °C; desolvation temperature, 210 °C; cone gas flow, 50 L/h; and desolvation gas flow, 600 L/h. Nitrogen gas was used for desolvation and for the cone.

Statistical Analysis. Samples were analyzed in triplicate, and a one-way analysis of variance was performed using SAS version 9.1. Fisher's least significant differences (LSD) at $P < 0.05$ was tested to find significant differences among samples.

Table 1. Anthocyanin Composition on a Dry Basis (mg/100 g) for Six Manitoba Berries[a]

fruit	wild blueberry	saskatoon berry	raspberry	strawberry	chokecherry	seabuckthorn
moisture %	85.71	75.25	88.61	87.53	66.83	81.38
Dp-3-glc	84.39 b	263.76a	86.49 b	0.14 c	0.10 c	0.05 c
Cy-3-gal	ND	ND	3.62 a	ND	0.16 b	0.22 b
Dp-3-rut	17.92 b	42.72 a	ND	1.12 c	1.10 c	0.08 d
Dp-3-gal	25.82 b	15.21 c	80.34 a	1.65 e	2.01 d	ND
Cy-3-glc	27.48 d	117.67a	35.88 c	9.53 e	46.01 b	0.05 f
Cy-3-rut	ND	0.34 c	80.37 b	ND	107.01 a	0.14 c
Pt-3-glc	30.34 a	17.26 b	15.21 c	ND	3.79 d	ND
Pt-3-gal	25.46 a	18.60 b	11.34 c	ND	8.12 d	ND
Mv-3-glc	139.60a	47.38 b	0.41 d	0.56 d	3.23 c	0.15 f
Pg-3-glc	29.56 b	ND	3.58 c	75.66 a	2.45 d	ND
Pn-3-glc	17.37 a	ND	1.01 c	4.49 b	ND	ND
Pn-3-gal	16.41 a	14.67 b	0.12 e	0.85 c	0.37 d	0.02 f
Pn-3-ara	1.11 c	3.14 b	10.34 a	ND	ND	0.06 d
Mv-3-gal	101.20a	14.30 b	0.21 d	0.32 d	1.73 c	0.03 e
Mv-3-ara	27.36 a	0.56 c	10.10 b	0.14 e	0.31 d	ND
others	14.31 b	6.75 c	26.19 a	3.56 d	1.24 e	0.04 f
total	558.33a	562.36a	365.21 b	97.46 d	177.39 c	0.84 e
LSD	3.45	2.02	1.89	5.21	4.34	3.22

[a] $P < 0.05$ using Fisher's least significance difference (LSD). Different letters (a, b, c, d, e, and f) in the same row are used to show significant differences among these fruits.

RESULTS AND DISCUSSION

Consumption of berries can provide a good source of essential micronutrients such as vitamin C and folic acid (*5, 6, 26*). Because berries are also rich sources of dietary phenolics known for potential multihealth benefits, six Manitoba berry extracts (wild blueberry, Saskatoon berry, raspberry, chokecherry, strawberry, and seabuckthorn) were studied for their anthocyanin compositions. Although the anthocyanin contents of berries have been previously reported (*12, 27–31*), there was no data on the phenolic composition of berries grown in Manitoba. Data on anthocyanin composition of berries grown in Manitoba will reveal the potential of these fruits on the regional and international markets. The study was conducted in response to recent interests on the nutritional and health benefits of Manitoba berries, especially Saskatoon berry, wild blueberry, and seabuckthorn. Another driving force is the functional food research and development for local crops aimed at the worldwide fruit market.

The fruit samples were freeze-dried upon receipt to minimize changes in the chemical compositions and other functionality of fruits. Removal of water from food products in order to increase their shelf life can be achieved by different dehydration techniques. Vacuum freeze-drying of biological products is the best method of water removal, with end products of the highest quality, as compared with other dehydration techniques (*32, 33*). Freeze-dried strawberries were found to be of excellent color and flavor, with high rehydration capacity. Freeze-dried blueberries had the highest retention of important components, soluble solids, and color when compared to berries dehydrated using other methods (*32*).

One advantage of this study was that the quantification of the anthocyanins was performed by HPLC and using anthocyanin references. Therefore, the quantification is based on different references and not by one single reference and presented as cyanidin-3-*O*-glucoside equivalents. However, some peaks were still unlabeled because of the unavailability of all standards. We selected nine major anthocyanin standards to measure key anthocyanins in the fruits. Fewer number of standards were reported in previous studies (*18, 34, 35*), and thus, a reasonable number of anthocyanins were selected for this study to identify individual anthocyanins present in fruit samples. Some peaks were not labeled because of the lack of corresponding anthocyanin standards. The unlabeled peaks were considered as anthocyanins because they had a maximum UV absorption at 520 nm. The total anthocyanins reported (**Table 1**) include both labeled and unlabeled HPLC peaks. In the present study, the extraction efficiency ranged from 80 to 100% for the anthocyanin standards. The recoveries were within the range previously reported (*17, 18, 20*). In the calculation of the final results, these recoveries were not taken into account because the identities of some peaks were unknown. The coefficient of variation (CV) for the repeatability of the HPLC injections was less than 10%. Although measuring the recovery % is useful for validating the method for extraction of anthocyanins, most studies make use of only statistical analysis (e.g., CV and P-value) to show the reproducibility of the extraction method (*5, 36, 37*).

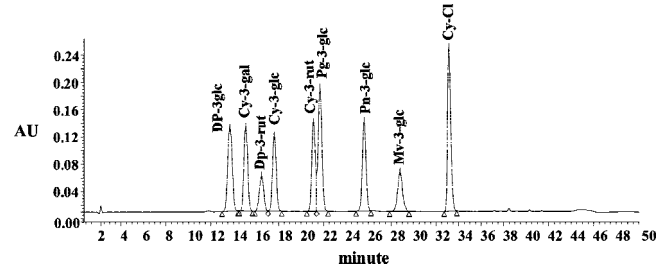

Figure 3. HPLC chromatograms (520 nm) for anthocyanin standards Dp-3-glc, Cy-3-gal, Dp-3 rut, Cy-3 glc, Cy-3-rut, Pg-3-glc, Pn-3-glc, Mv-3-glc, and Cy-Cl.

Anthocyanin standards were run to confirm the presence of these phenolics in each fruit sample. **Figure 3** shows the HPLC chromatogram of anthocyanin standards. Dp-3-glc showed the shortest retention time (RT, 13.9 min), whereas Cy-Cl had the longest RT (33.1 min). The UV profile at 520 nm was used to distinguish anthocyanins from other phenolics. The HPLC chromatogram of wild blueberry (**Figure 4**) was selected as an example to illustrate the separation of anthocyanins present in all berry fruits. The results were reported on the dry weight basis (mg/100 g) of fruits; however, data on moisture content is included to enable conversion of anthocyanin content to the fresh weight basis. Saskatoon berry and wild blueberry showed a high content of total anthocyanins (562.4 and 558.3 mg/100 g, dry basis)

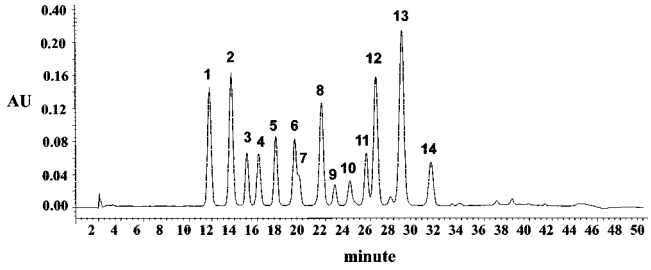

Figure 4. HPLC chromatogram (520 nm) for anthocyanins present in wild blueberry. (1) Dp-3-gal, (2) Dp-3-glc, (3) Dp-3-rut, (4) Cy-3glc, (5) Pt-3-glc, (6) Pt-3-gal, (7) Cy-3-rut, (8) Pg-3-glc, (9) Pn-3-gal, (10) Mv-3-gal, (11) Pn-3-glc, (12) Pn-3-ara, (13) Mv-3-glc, (14) Mv-3 ara.

that were not significantly ($P > 0.05$) different from each other (**Table 1**). Other anthocyanins reported in wild blueberry include Mv-3-ara, Pt-3-glc (*28*), Dp-3-gal, and Dp-3-ara (*31*). The corresponding values for other berries are as follows: raspberry (365.2 mg/100 g), chokecherry (177.19 mg/100 g), and strawberry (97.5 mg/100 g); they were significantly different ($P < 0.05$) from each other. The anthocyanin content of seabuckthorn was negligible (0.84 mg/100 g) (**Table 1**). Saskatoon berry and wild blueberry could rank as high anthocyanin berries, raspberry and chokecherry as medium anthocyanin berries, strawberries as low anthocyanin berries, and seabuckthorn as negligible anthocyanin berries. A total of 15 anthocyanins were identified from Manitoba berries, although nine major anthocyanin standards were used to identify individual anthocyanins (**Table 1**). This was performed using LC-MS and LC-MS/MS as well as information from literature (*28*, *38*).

Dp-3-glc was 263.8 (mg/100 g) in Saskatoon berry and 84.4 (mg/100 g) in wild blueberry. The levels of Mv-3-glc in Saskatoon berry and wild blueberry were 47.4 and 139.6 (mg/100 g), respectively (**Table 1**). Raspberry, strawberry, and chokecherry contained mainly Cy-3-rut, Cy-3-glc, and Pg-3-glc. The content of Cy-3-rut in chokecherry and raspberry was 107.0 and 80.4 (mg/100 g), respectively, and strawberry did not show the presence of any Cy-3-rut. Among the reddish berries, chokecherry showed a higher content of Cy-3-glc (46.0 mg/100 g) than raspberry (35.9 mg/100 g) and strawberry (9.5 mg/100 g). In contrast, strawberry had a higher level of Pg-3-glc (75.7 mg/100 g) than chokecherry and raspberry. In the present study, the bluish berries such as wild blueberry and Saskatoon berry seemed to exhibit a higher amount of Dp-3-glc and Mv-3-glc (**Table 1**) when compared to other berries. On the other hand, reddish berries (raspberry, strawberry, and chokecherry) showed a higher amount of Cy-3 rut, Cy-3-glc, and Pg-3-glc than wild blueberry and Saskatoon berry (**Table 1**). Delphinidin has been reported to be responsible for the bluish colors, whereas cyanidin and pelargonidin were responsible for red and purple colors (*39*), respectively. Cyanidin, delphinidin, and pelargonidin are the most common anthocyanins in nature, with cyanidin glycosides reportedly present in nearly 90% of all fruits (*40*). Anthocyanin content in fresh berry fruits has been reported in wide ranges from 10 to 600 mg/100 g (*15*, *16*). Published values (mg/100 g) include 63–484, 8–79, 210–400, and 305–631 for wild blueberry, strawberry, raspberry, and chokecherry, respectively (*15*, *16*, *22*). Therefore, the anthocyanin levels found in Manitoba fruits fall within the literature values, with the exception of chokecherry, which had lower levels.

UPLC-MS/MS equipped with an electrospray ionization source was used to confirm the structure of each anthocyanin compound in fruit samples. **Figure 5** shows MS the spectra of individual anthocyanin standards in the positive ESI mode. All mass spectra data indicated anthocyanins were presented as their glycoside forms (**Table 2**). No aglycones were observed in the fruit samples.

The major mass fragmentation ions of anthocyanins present in berries are associated with their corresponding aglycones (**Table 2**). For example, ions at *m*/*z* 287 and 449 suggest that the aglycone is cyanidin (*m*/*z* 287) (**Figure 5**, panels **b** and **d**, respectively). The parent ion (*m*/*z* 449) shows the addition of glucose or galactose (*m*/*z* 162) to cyanidin. Mass spectrometry cannot distinguish between them, and thus, the retention time of each standard in HPLC was used for their identification (**Figure 2**). Another example of anthocyanin in which mass fragmentation ions were measured is Pn-3-glc. The mass of its parent ion was *m*/*z* 463, and its daughter fragment had a value of *m*/*z* 301 (**Figure 5g**). The ion at *m*/*z* 301 corresponded to methylation of cyanidin ($287 + 14 = 301$) in Pn-3-glc. Overall, the MS results showed the major aglycones corresponding to each anthocyanin glycoside (**Table 2**). These included pelargonidin (*m*/*z* 271), cyanidin (*m*/*z* 287), peonidin (*m*/*z* 301), delphinidin (*m*/*z* 303), and malvidin (*m*/*z* 331). The mass spectra of the anthocyanins present in fruit samples showed the same fragmentation patterns as those found in standards and thus supported the identification of each compound (**Table 2**).

For this study, it was important to observe the level of anthocyanins that contribute to the pigments of these fruits with small variation between each analysis. To achieve these aims, the fruits from one variety were randomly picked from several trees at their peak of ripeness (e.g., color and softness) during the period from June to August of 2006 and then combined for further analyses. It has been reported that factors such as genes, soil type, light, temperature, and agronomic conditions affect the total anthocyanins composition in plants (*24*, *39*). Therefore, the phenolic contents of fruits may vary from batch-to-batch and from year-to-year (*24*, *26*, *39*). Up to now, there is little published literature about phytochemical and antioxidant activity values of Manitoba fruits. However, the USDA database includes nutritional and phytochemical content of some fruits (*26*, *41*, *42*). Our recent study showed that the total proanthocyanidin contents of Manitoba berry fruits varied between 275.55 and 504.77 mg/100 g (*7*). Furthermore, these fruits were demonstrated to have good antioxidant capacities (*7*).

The efficiency of separation can be achieved by elevating the column temperature. Three different temperatures (23, 35, and 40 °C) were used, and 35 °C was found to be the optimum temperature for anthocyanins analysis. When the column was held at room temperature, almost all anthocyanins overlapped and appeared as a large, single peak in an HPLC chromatogram. There was no difference in the separation when column temperature was held at 35 or 40 °C. Therefore, 35 °C was selected for further analysis. Use of formic acid showed higher resolution when compared to previously reported acetic acid (*25*). Also, increasing the formic acid concentration up to 6% resulted in a higher resolution when compared to a concentration of 4.5%, as suggested in the previous reports (*18*).

Berries are reported to have potential antioxidant, anticarcinogenic, and anti-inflammatory properties and, thus, valuable health benefits (*5*, *6*, *8–10*). This study suggests that anthocyanins can be extracted from Manitoba fruits and can be used for nutraceutical and functional food purposes. Manitoba berries

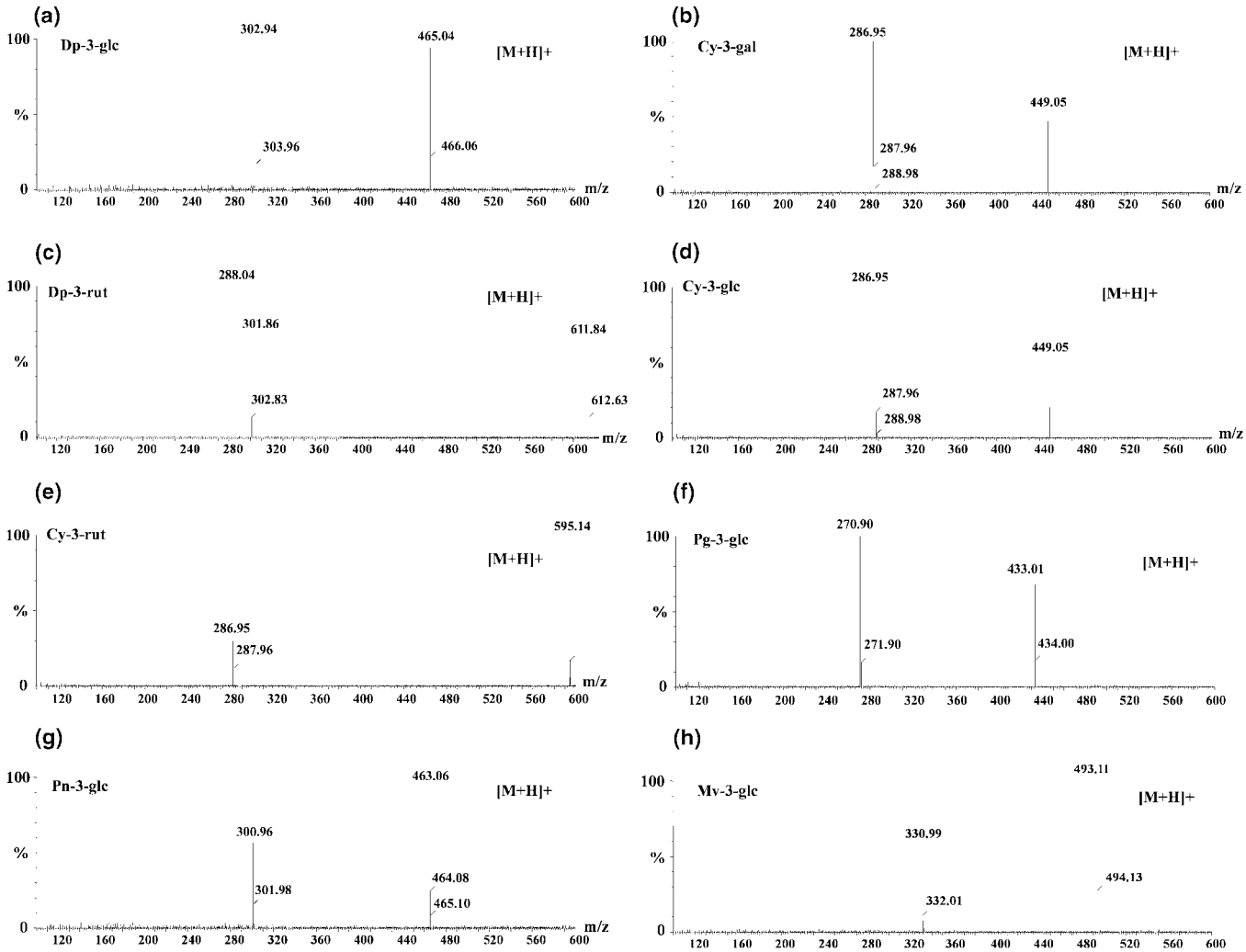

Figure 5. Mass spectra (*m*/*z*) for anthocyanin standards Dp-3-glc, Cy-3-gal, Dp-3 rut, Cy-3-glc, Cy-3-rut, Pg-3-glc, Pn-3-glc and Mv-3-glc.

Table 2. Mass Spectra (*m*/*z*) of Anthocyanins Present in Manitoba Berries

anthocyanin	RT (min)	major ions (*m*/*z*) $[M + H]^+$
Dp-3-gal	11.7	465, 303
Dp-3-glc	13.7	465, 303
Cy-3-gal	15.1	449, 287
Dp-3-rut	16.5	611, 303
Cy-3glc	17.6	449, 287
Pt-3-glc	19.3	479, 317
Pt-3-gal	19.6	479, 317
Cy-3-rut	21.3	595, 287
Pg-3-glc	21.6	433, 271
Pn-3-gal	22.8	463, 301
Mv-3-gal	24.1	493, 331
Pn-3-glc	25.6	463, 301
Pn-3-ara	26.4	449, 317
Mv-3-glc	28.8	493, 331
Mv-3 ara	31.3	463, 331

can also be added as natural antioxidants in a wide range of foods, thereby enhancing their shelf life as well as their appearances.

This study demonstrated that Saskatoon berries and wild blueberries have high levels of total anthocyanins. Saskatoon berries have been used as a native prairie fruit crop. There are now more than 3000 acres of Saskatoon berries planted in Saskatchewan, Manitoba, and Alberta (*43*). Seabuckthorn, reported to have a high content of vitamin C (4.2–13.2 g/L) (*44*), had negligible amounts of anthocyanins. This study suggests that growing berries, particularly Saskatoon berries and wild blueberries, is important for agricultural production of unique, anthocyanin-enhanced fruits and for value-added programs on functional foods and nutraceuticals.

In conclusion, six Manitoba berry extracts were studied for their anthocyanin compositions. These berries were wild blueberry, Saskatoon berry, raspberry, chokecherry, strawberry, and seabuckthorn. The total anthocyanin content of Manitoba berries ranged from 0.3 to 562.4 (mg/100 g). Saskatoon berry and wild blueberry showed the highest level of anthocyanins, and seabuckthorn showed the lowest level of these phenolics. The high anthocyanin content in Saskatoon berry and wild blueberry could enhance the production of berries in prairie regions of Canada. Eight key major anthocyanins were isolated from Manitoba berries, including Dp-3-glc, Cy-3-glc, Cy-3-gal, Dp-3-rut, Cy-3-rut, Pg-3-glc, Pn-3-glc, and Mv-3-glc. UPLC-MS/MS equipped with an electrospray ionization source was useful for confirming the structure of each anthocyanin compound in fruit samples. The mass spectra of the anthocyanins present in fruit samples showed the same fragmentation patterns as those found in standards and, thus, supported the identification of each compound. The type and the content of the above anthocyanins varied for each berry. Bluish berries, including Saskatoon berry and wild blueberry, contained higher amounts of Dp-3-glc and Mv-3-glc. Reddish berries, including raspberry, strawberry and chokecherry, contained higher amounts of Cy-3-glc and Pg-3-glc. Our study demonstrated that Manitoba

berries can benefit the agricultural and food industries by contributing valuable phytochemical constituents.

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